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Effects of sleep deprivation on pain-related factors in the temporomandibular joint

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ABSTRACT

Background: The objective of this study was to investigate the effects of experimental sleep deprivation (SD) on the temporomandibular joint (TMJ) in rats by examining pain-related factors and to determine the possible involvement of estrogen and NF (nuclear factor) κB signaling in the TMJ synovial membrane.

Methods: The influence of SD, conducted in rats using the modified multiple platform method, was estimated by observing behavioral manifestations and examining changes in serum hormone levels. The morphologic changes of synovial tissue were observed with light microscopy and the serum levels of estrogen were measured by radioimmunoassay. Activation of NF-κB in the synovial membrane was examined using an immunofluorescence technique, and the expression levels of interleukin (IL) 1β, IL-6, tumor necrosis factor α, cyclooxygenase 2, and inducible nitric oxide synthase were measured with real-time polymerase chain reaction.

Results: The SD group showed evidence of elevated anxiety and stress, and increased plasma levels of estradiol compared with the control group. The activity of NF-κB was significantly enhanced and translocation of NF-κB p65 was evident in the synovial membrane after SD. The expression of pain-related factors IL-1β, IL-6, cyclooxygenase-2, tumor necrosis factor α, and inducible nitric oxide synthase in the synovial membrane significantly increased after SD.

Conclusions: These results indicate that SD increases serum levels of estrogen and induces alterations in pain-related factors in the TMJ. The NF-κB pathway has been associated with the regulation of these inflammatory cytokines and plays an important role in temporomandibular disorders.

1. Introduction

Recently, as the treatment of temporomandibular disorders (TMDs) has transformed from a biomedical model into a biopsychosocial medical model, the role of psychological factors in TMD pathogenesis has received increasing attention [1,2]. It has been suggested that psychological factors are closely related to the occurrence, development, and effective

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treatment of TMD [3,4], with increasing numbers of scholars arguing that TMD is a psychosomatic disease [5–7]. Aside from psychological stress, additional factors that can influence TMD include gender [8], age [4], depression [9], sleep problems [10], and general health [5].

Because sleep disorders were discovered to be a probable factor in the development of TMD, many studies have started to focus on the association between sleep quality and TMD through retrospective analyses of clinical data [11–15]. Many studies have indicated that TMD patients generally suffer with poor sleep quality [1,16]. However, there have been few reported well-controlled experiments investigating whether poor sleep quality can cause the pathologic changes associated with the temporomandibular joint (TMJ). Sleep deprivation (SD) can lead to immune system incompetence [17–19] and may also induce psychological stress [1,2].

TMDs are an assorted set of clinical conditions characterized by pain in the TMJ and/or masticatory muscles. Joint inflammation is thought to be a major cause of pain in patients with TMDs [3,20–22]. Estrogen plays an important role in joint inflammation among various other contributing factors [23]. The ratio between female and male patients with TMD is 8–10:1, and the two peak periods of incidence are in puberty and during menopause [24]. Recent studies show that estrogen receptors have been detected in TMJ tissue [25]. Furthermore, 17β-estradiol can lead to the nuclear translocation of nuclear factor (NF) κB, which can subsequently upregulate transcription of tumor necrosis factor (TNF) α, interleukin (IL) 1, IL-6, IL-8, cyclooxygenase (COX) 2, and inducible nitric oxide synthase (iNOS) [26].

NF-κB plays a pivotal role in regulating inflammation-associated genes and may exacerbate TMJ pain through the aggravation of synovial inflammation, which may be a possible local mechanism underlying the effects of estradiol on TMJ inflammation and pain [27,28].

In this study, we induced SD in rats by applying the modified multiple platform method (MMPM) [29]. In addition, we included a tank control (TC) group in which rats were free of SD. Our aim was to determine whether SD would: increase the serum concentration of estrogen, lead to the activation of NF- κB and changes in pain-related factors, and induce the inflammation of the TMJ. Thus, we explored whether the effects of estrogen were facilitated by potentiation of the NF-κB pathway. To our knowledge, this study is the first to investigate whether SD can induce alterations in pain-related factors in the TMJ, and examine the possible involvement of estrogen and NF-κB signaling in the TMJ synovial membrane.

2. Material and methods

2.1. Animals

This study used 120 female Wistar rats (8 wk old, 210–230 g), which were purchased from the Animal Center of Shandong University (Jinan, China). All rats had undergone a 2-wk acclimatization in laboratory conditions before the experiment began, with free access to food and tap water. The animal experiment was carried out in the animal experiment center of Jinan Military General Hospital. The rats were housed under controlled temperatures (21°C–23°C), with a 12:12 h light–dark cycle. The water in the tank was changed daily throughout the study period. The experimental protocols were approved by the Animal Use and Care Committee of Jinan General Military Hospital and were in line with the Ethical Guidelines of the International Association for the Study of Pain.

Table – Animal experimental group design.

Group	Duration of SD					
	0 d (CC)	1 d	3 d	5 d	7 d	9 d
TC	10	10	10	10	10	10
SD	10	10	10	10	10	10

Data values represent numbers of rats.

2.2. Apparatus

We used a SD model provided by the animal experiment center of Jinan Military General Hospital. Other equipment used in the study included a confocal laser scanning microscope (TCS-SP5; Leica, Solms, Germany), high-speed centrifuge (Sigma I-14; Tiancheng Technological, Shanghai, China), spectrophotometer (Nanodrop 2000; Thermo Fisher Scientific, Beijing, China), polymerase chain reaction (PCR) apparatus (Realplex 4, Eppendorf, Hamburg, Germany), Access Immunoassay System (Beckman Coulter, Beijing, China), and tissue dissociator (gentleMACS; Miltenyi Biotec, Cologne, Germany).

2.3. Animal grouping

We randomly divided the rats into a TC group and a SD group. The backs of the rats were dyed different colors to distinguish the groups. Each group was divided into six time-groups according to the duration of the SD (0, 1, 3, 5, 7, and 9 d), with 10 rats in each time-group (Table). The rats with 0 d SD acted as the normal cage controls (CC rats). The CC and TC groups were regarded as the control group. All the rats were fed normally and supplied with water. The rats underwent a 1-wk period of acclimatization in laboratory conditions before the experiment began.

2.4. Model establishment

SD was conducted using the MMPM as described in the articles by Suchecki *et al.* [29–31], with minimal modification (Fig. 1). The TC group was placed in the tank (left panel) with a grid floor (125.0 cm × 84 cm). Each group of SD rats was placed in the container (right panel) with 15 narrow platforms (6.3 cm in diameter). The rats could thus move around inside the tank by jumping from one platform to another. The tanks were filled with water until the water level was 1 cm below the upper surface of the platforms. When the rats reached the paradoxical phase of sleep, muscle atonia set in and they fell into the water and woke. The normal CC rats were maintained in cages in the same room as the SD and TC rats.

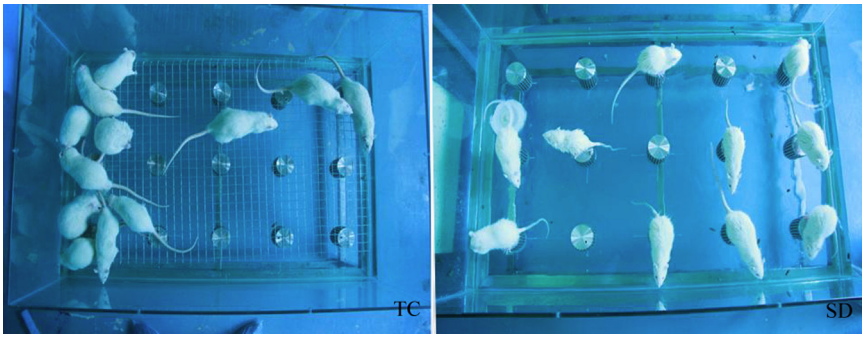

Fig. 1 – Experimental model of TC group and SD group. Left panel: tank with a grid which permitted the animals not to fall in the water. Right panel: tank with 15 narrow platforms. The rats could move around inside the tank by jumping from one platform to another. The tanks were filled with water until the water level was 1 cm below the upper surface of the platforms. (Color version of figure is available online.)

2.5. *Hormonal determination*

For all rats, blood samples were collected from the cardiac ventricle at each time point. The SD and TC rats were sacrificed after 1, 3, 5, 7, or 9 d according to their groups, and the CC rats were sacrificed at the same time as the 9 d SD group rats. This was done under anesthesia with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight). The plasma was then separated by centrifugation (3000*g*, 15 min, 4°C) and stored immediately at −20°C for subsequent hormone testing. Each hormone assay was carried out on the same day. The serum levels of 17β-estradiol were measured by radioimmunoassay using an Access Immunoassay System (Beckman Coulter, CA).

2.6. *Tissue preparations*

The synovial membrane of the TMJ is composed of a layer of synovial lining and a layer of connective sublining [32]. The TMJ synovial membrane was bilaterally harvested from five rats per group for ribonucleic acid and nuclear extractions. The membranes of the other five rats in each group were fixed in 10% buffered paraformaldehyde overnight at 4°C. The specimens were dehydrated in graded alcohols and xylene, embedded in paraffin, and sectioned sagittally at a thickness of 5 μm.

2.7. *Immunofluorescence histochemistry*

After deparaffinization, rehydration, and disposal of the 3% hydrogen peroxide, the pressure cooking method was used for antigen retrieval. Antigens were retrieved by boiling the TMJ sections in 0.01 mol/L citrate buffer (pH 6.0) for 2 min. Sections were blocked with 0.5% Triton X-100 for 30 min at room temperature and blocked in a 5% nonfat milk-Tris-buffered saline and Tween 20 solution for at least 30 min in case of nonspecific protein staining. They were then washed with phosphate buffered saline (PBS) and incubated with anti NF-κB P65 primary antibodies (1:100) (Cell Signaling, Boston) overnight at 4°C. After extensive washing with PBS, the sections were incubated with Alexa Fluor 488 (1:100; donated by Professor Yaping Wu (Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, the Netherlands), goat anti-mouse secondary antibody and Hoechst 33,342 for 60 min at room temperature. They were again washed with PBS, and then covered with 2% Mowio fluorescence mounting medium coverslips. Confocal microscopic images were acquired using a Leica scanning microscope (TCS-SP5), and the images were processed using LSM 5 Release 4.2 software (Leica Microsystems, Wetzlar, Germany).

2.8. *Real-time reverse transcription-polymerase chain reaction analysis*

The synovial membranes of the TMJ were homogenized in gentleMACS Dissociator (Miltenyi Biotech). Ribonucleic acid was then isolated with Trizol Reagent (Invitrogen-life Technologies, Carlsbad), and reverse-transcribed at 50°C for 30 min. Real-time reverse transcription PCR was performed using the Quant One Step quantitative reverse transaiption-polymerase chain reaction (SYBR Green I) Kit (FP303; Tiangen Biotech Corporation, Beijing, China). The PCR reaction conditions were as follows: reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 2 min, denaturation at 94°C for 20 s, annealing at 50°C–60°C for 20 s, and extension at 68°C for 20 s. We used the same internal reference (glyceraldehyde 3-phosphate dehydrogenase) for TNF-α, IL-1β, IL-6, COX-2, and iNOS.

The primers were designed as follows: TNF-α forward (5′-3′: CCAGGTTCTCTTCAAGGGACAA), reverse (5′-3′: CTCCTGG TATGAAATGGCAAATC); IL-1β forward (5′-3′:CTCGTGCTG TCTGACCCAT), reverse (5′-3′: CAAACCGCTTTTCCATCTTC); IL-6 forward (5′-3′: CCAAGACCATCCAACTCATCTTG), reverse (5′-3′:CACAGTGAGGAATGTCCACAAAC); COX-2 forward (5′-3′:GGTGTCCCTTCGCCTCTT), reverse (5′-3′: CAGTTGAACG CCTTTTGATTAG); iNOS forward (5′-3′: TGGAGCGAGTTGTGG ATTG), reverse (5′-3′: AACCTCTGCCTGTGCGTC); glyceraldehyde 3-phosphate dehydrogenase forward (5′-3′: CACGGCAAG TTCAACGGCACA), and reverse (5′-3′: AGCGGAAGGGGCGGAG ATG).

2.9. *Statistical analysis*

Statistical analysis was performed with SPSS version 16.0 for Window XP. All data were presented as means ± standard error. Differences between groups were analyzed by one-way

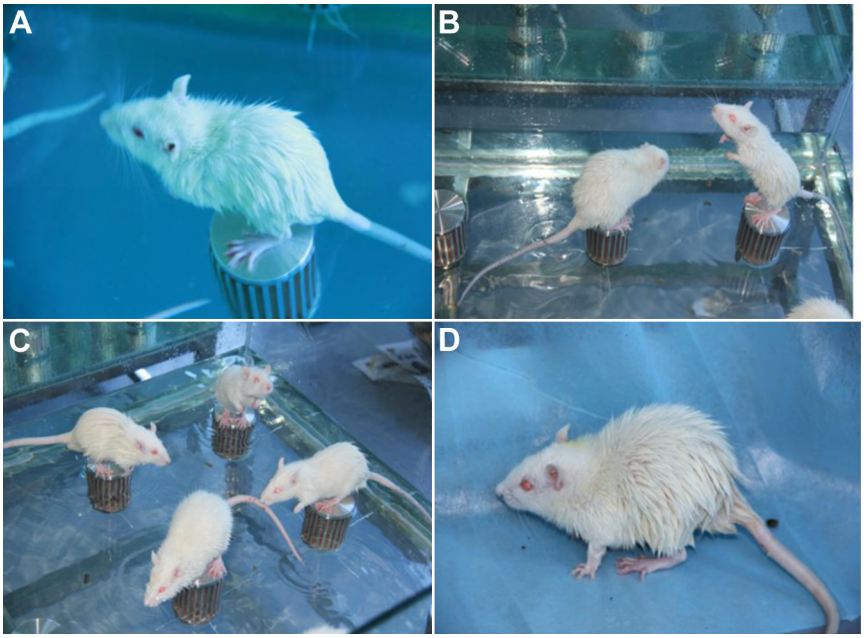

Fig. 2 – Behavioral changes before and after SD. Rats presented poor mental state and physical weak body after SD. Their fur appeared rough and disheveled, and they lagged in response. At the early stage of SD, the rats showed a rising excitability and responded quickly to surrounding stimuli. On day 5 of SD, all SD group rats showed the phenomenon of "irritation". On the seventh day, all SD rats showed an extremely weak performance. The behavioral performance of narrow platform rats on day 0 (A), day 1 (B), day 5 (C), and day 9 (D). (Color version of figure is available online.)

analysis of variance. P values <0.05 were considered statistically significant.

3. Results

3.1. Behavioral changes

As shown in Figure 2, the rats in the SD group presented poor mental state and physical weak body after SD. Their fur appeared rough and disheveled, and they lagged in response. The rats were observed falling into the water from time to time, and the frequency of this increased as the duration of SD increased. At the early stage of SD, the rats showed a rising excitability and responded quickly to surrounding stimuli. On day 5 of SD, all SD group rats showed the phenomenon of "irritation": given the stimulus of touch, the rats displayed obvious aggressive behaviors including screaming, escaping, biting, and fighting with each other. On the seventh day, all SD rats showed an extremely weak performance: they were listless and trembling; they had an accelerated respiratory rate; they had reduced locomotor activity, were hunched over, displayed astasia, and found it difficult to move forward; they were indifferent to environmental stimulation; and their daily diet was poor.

3.2. Histopathologic evaluation of TMJ synovial membrane

As shown in Figure 3, the histopathologic features of the synovial membrane in the control group appeared to be normal, displaying tight-packed polygon synovial cells with large nuclei (Fig 3A). In the 1 d SD group, the cell arrangement was looser (Fig 3B). In the 3 d SD group, there was hemangiectases of capillaries, edema of the sublining layer, and increased macrophages (Fig 3C). In the 5, 7, and 9 d SD groups, synovitis and intercellular edema were evident, with small blood, infiltration by macrophages, and synovial lining hyperplasia (Figs. 3D–F).

3.3. Effect of SD on serum levels of estradiol

As shown in Figure 4, the serum levels of estradiol in the SD group were markedly increased compared with those in the TC group. (The following data are expressed as mean ± standard error.) The concentrations of estradiol were 26.109 ± 0.51 ng/mL, 31.204 ± 1.27 ng/mL, and 33.422 ± 1.25 ng/mL in the SD group on days 1, 3, and 5 of SD, respectively. The levels of estradiol were highest in the 5 d SD group. These values were markedly increased compared with those of the 0 d SD group (23.284 ± 1.22 ng/mL, $P<0.01$). The levels of estradiol in the 7 d SD group had returned to the control group levels ($P>0.05$). In contrast, during the same periods (on days 1, 3, and 5), the serum concentrations of estradiol in the TC group were 23.105 ± 1.81 ng/mL, 24.434 ± 1.89 ng/mL, and 23.429 ± 1.19 ng/mL, which were significantly lower than those in the SD group ($P<0.05$).

3.4. Activation of NF-κB in the synovial membrane of inflamed TMJs

To evaluate activation of the NF-κB pathway in the synovial membrane of inflamed TMJs, P65 was examined with confocal

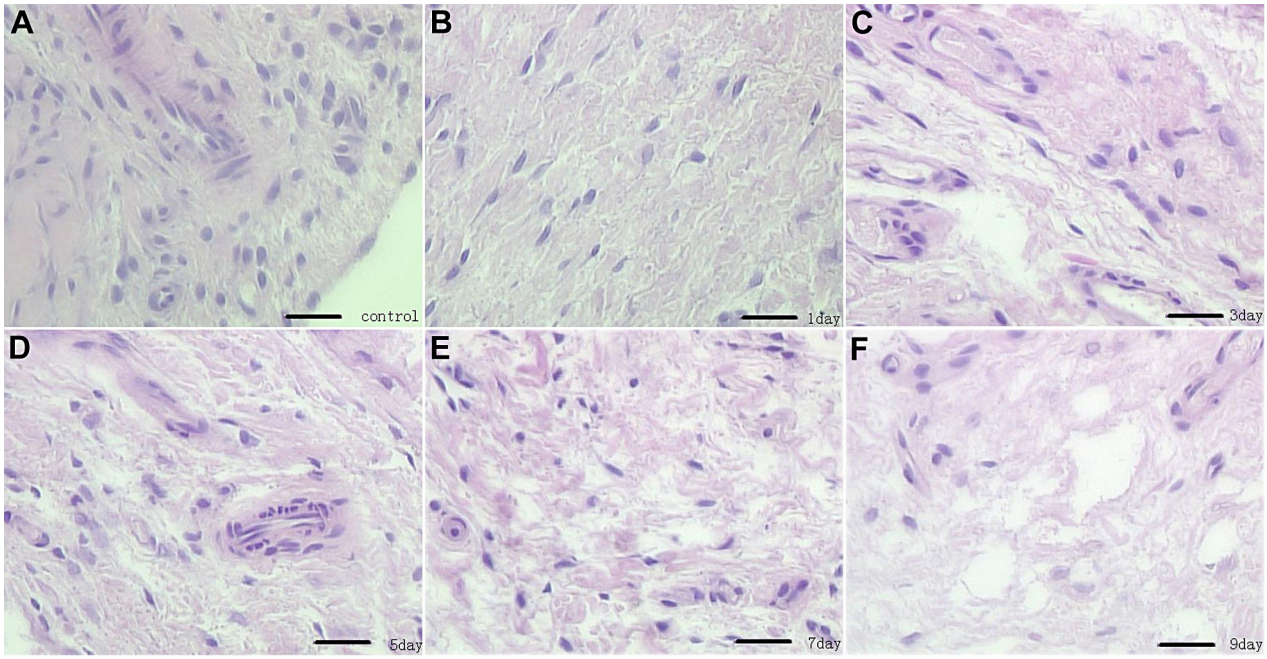

Fig. 3 – Microstructural changes in TMJ synovial membrane induced by SD: representative photomicrographs (×400) of the TMJ region in all rat groups. (A) Control group: synovial membrane appeared normal, tight-packed polygon synovial cells with large nuclei. (B) First day after SD: cell arrangement was looser. (C) Third day after SD: there was hemangiectases of capillaries, edema of sublining layer, and increased macrophages in synovial membrane. (D, E, and F) each represent fifth day, seventh day, and ninth day after SD: synovitis and intercellular edema were evident, with small blood, infiltration by macrophages, and synovial lining hyperplasia. (Color version of figure is available online.)

laser scanning microscopy. As shown in Figure 5, in the control group, 1 d and 3 d SD groups, the fluorescence A488 signal of p65 was located in the cytoplasm of cells in the synovial membrane (including the connective sublining layer) (Figs 5A,B,E and F). After the SD, in the 5 and 7 d SD groups, the fluorescence signal of p65 was mainly located in the nuclei of synovial cells (Figs. 5C,D,G and H).

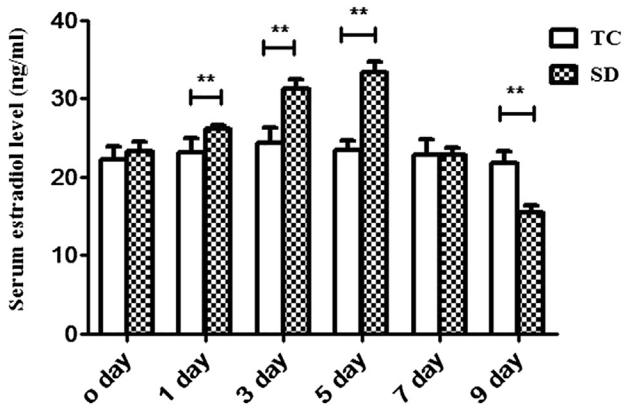

Fig. 4 – Comparison of serum estradiol levels in the SD group and TC group. The levels of serum estradiol in the rats of both the TC group and SD group changed as the duration of SD increased. The SD group showed more distinct changes: the level of estradiol increased and peaked on day 5, then dropped on days 7 and 9. Meanwhile, these values of SD group on days 1, 3, and 5 were markedly higher than those of TC group at the same time point ($P < 0.01$). (note: ** refers to $P < 0.01$)

3.5. *The messenger ribonucleic acid levels of pain-related factors in synovial membrane of the TMJ*

As shown in Figure 6, The expression levels of IL-6 (A) in the SD group on days 3, 5, and 7 of SD were 1.997 ± 0.169, 2.419 ± 0.157, and 3.496 ± 0.118, respectively. (The following data are expressed as mean ± standard error of the mean.) The levels of IL-6 in the 7 d SD group were highest. These values were significantly different from each other ($P < 0.01$). The levels of IL-6 in the 9 d SD group had apparently decreased. In contrast, during the same periods (days 3, 5, and 7), the levels of IL-6 in the TC group were 1.589 ± 0.074, 1.504 ± 0.182, and 1.597 ± 0.146, which were significantly lower than those in the SD group ($P < 0.05$).

The expression levels of IL-1β (B) in the SD group were markedly increased compared with those in the TC group. The expression of IL-1β in the SD group on days 3, 5, and 7 of SD were 0.543 ± 0.0125, 0.632 ± 0.0734, and 0.940 ± 0.0809, respectively. The levels of IL-1β in the 7 d SD group were highest. These values were significantly different from each other ($P < 0.01$). The levels of IL-1β in the 9 d SD group had returned to the 1 d SD group levels ($P > 0.05$). In contrast, during the same periods (on days 3, 5, 7, and 9), IL-1β in the TC

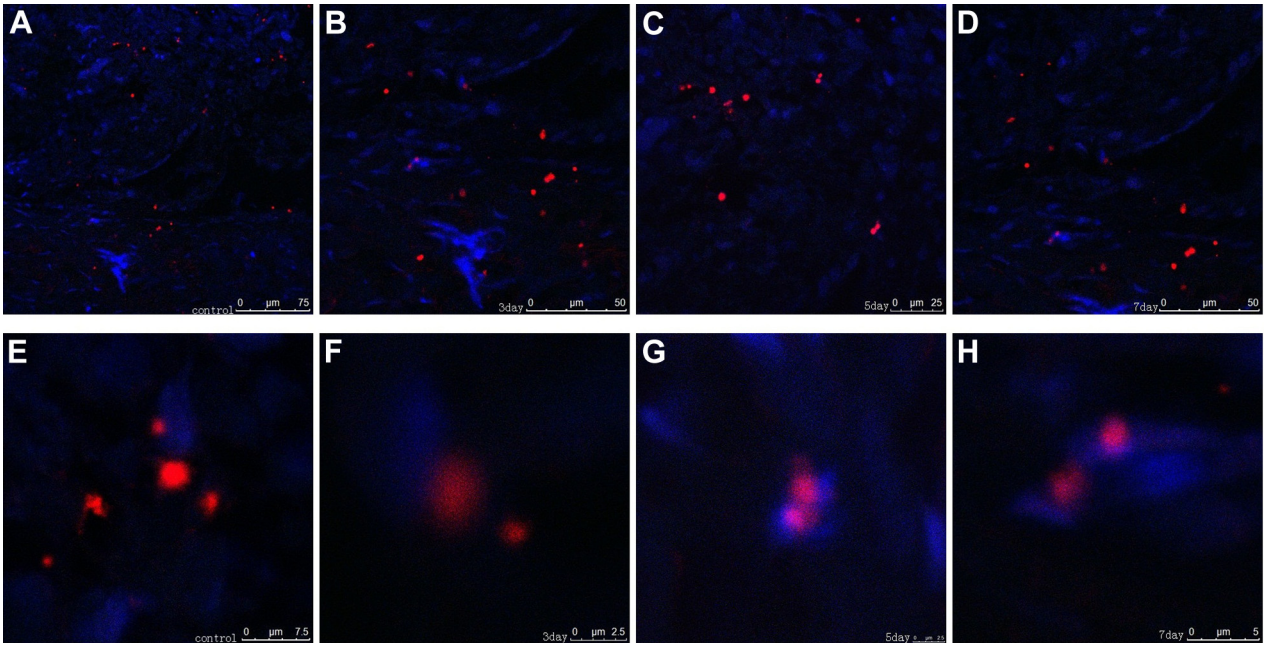

Fig. 5 – The translocation of NF-κB p65 in the synovial membrane of inflamed TMJs. The subcellular translocation of p65 was examined using confocal laser scanning microscopy. (E–H) were higher magnification of upper area in (A–D) correspondingly. In the control group (A and E) and 3 d SD group (B and F), the fluorescence A568 signal of NF-κB p65 (red) was located in the cell cytoplasm of the synovial membrane and the connective sublining layer. However, NF-κB p65 staining was located in the nuclei of the synovial cells in the 5 d SD group (C and G), and translocated into the nuclei of the synovial cells in the 7 d SD group (D and H). Nuclei were stained with Hoechst 33,342 (blue). The red staining of NF-κB p65 changed into watery blue after merging with the blue staining of the nuclei. (Color version of figure is available online.)

group were 0.472 ± 0.024, 0.485 ± 0.053, 0.467 ± 0.0489, and 0.456 ± 0.0314, which were significantly lower than those in the SD group ($P < 0.05$).

The expression levels of TNF-α in the SD group on days 3, 5, and 7 of SD were 1.746 ± 0.0323, 2.344 ± 0.137, and 2.782 ± 0.101. These values were significantly different from each other ($P < 0.01$). The levels of TNF-α in the 9 d SD group had returned to the 1 d SD group levels ($P > 0.05$). In contrast, during the same periods (days 3, 5, and 7), the levels of TNF-α in the TC group were 1.481 ± 0.117, 1.421 ± 0.0538, and 1.484 ± 0.092, which were significantly lower than those in the SD group ($P < 0.05$).

The expression levels of COX-2 in the SD group on days 3, 5, 7, and 9 of SD were 0.543 ± 0.012, 0.582 ± 0.0434, 0.810 ± 0.051, and 0.833 ± 0.0479. These values were significantly different from each other ($P < 0.01$). In contrast, during the same periods (days 3, 5, 7, and 9), the levels of COX-2 in the TC group were 0.485 ± 0.043, 0.467 ± 0.0488, 0.456 ± 0.0313, and 0.474 ± 0.0120, which were significantly lower than those in the SD group ($P < 0.05$).

The expression levels of iNOS in the SD group on days 3, 5, and 7 of SD were 1.226 ± 0.0496, 1.525 ± 0.0615, and 1.639 ± 0.0303. These values were significantly different from each other ($P < 0.01$). In contrast, during the same periods (days 3, 5, and 7), the levels of iNOS in the TC group were 1.114 ± 0.0495, 1.123 ± 0.0615, and 1.145 ± 0.0303 which were significantly lower than those in the SD group ($P < 0.05$).

Although the expression levels of pain-related factors mentioned previously in the TC group also increased compared with the CC group, the increases were less substantial than those of the SD group ($P > 0.05$, data not shown).

4. Discussion

To our knowledge, this is the first study to report significant changes in serum levels of estrogen and enhanced expression of pain-related factors in the synovial membrane of the TMJ, and the first to have investigated the molecular mechanisms implicated in these changes, which occurred after exposure to SD. In our study, we confirmed that SD could induce increases in the serum level of estradiol and synovitis, and intercellular edema in the synovial membrane of the TMJ. These changes corresponded to the translocation of NF-κB p65 and the expression of the pain-related factors IL-1β, IL-6, COX-2, TNF-α, and iNOS in the synovial membrane after SD.

In this study, we successfully established a SD model of rats using the MMPM. The MMPM is an effective means of depriving rats of paradoxical sleep (PS), wherein rats are placed onto several narrow platforms (6.3 cm in diameter) that are immersed in water, which avoids the additional stresses induced by both social isolation and movement restriction. When the rat enters PS, which is characterized by muscle

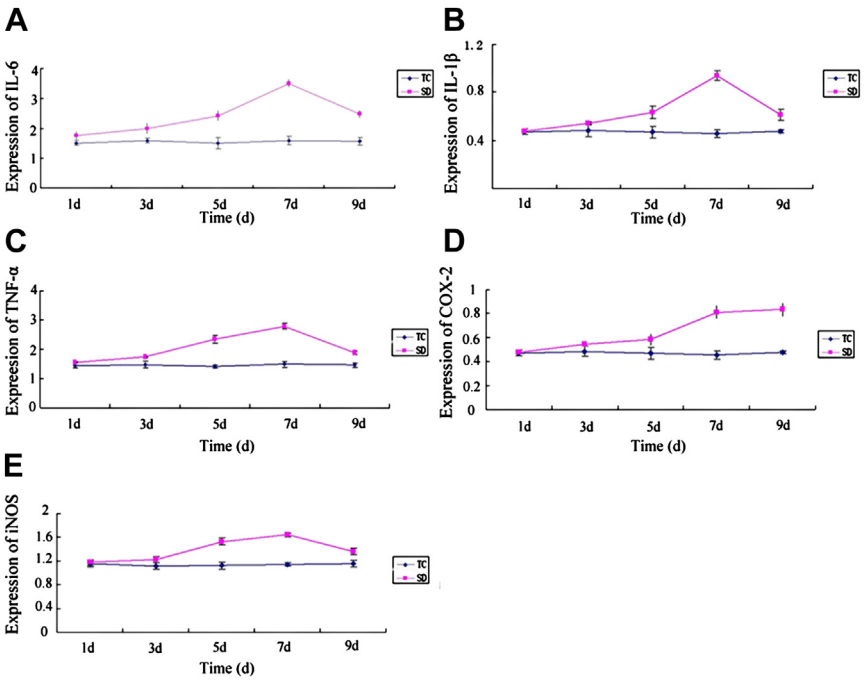

Fig. 6 – Expression of pain-related factors in the synovial membrane of the TMJ. Transcription of IL 6 (A), IL-1β (B), TNF-α (C), COX-2 (D), and iNOS (E) in the synovial membrane was examined by real-time fluorescent quantitative reverse transcription PCR, and differences were examined using one-way analysis of variance. IL-6: there were differences at days 1, 3, 5, and 7 of SD ($P < 0.01$). IL-1β: there were differences at days 3, 5, and 7 ($P < 0.01$). TNF-α: there were differences at days 3, 5, and 7 ($P < 0.01$). COX-2: there were differences at days 3, 5, 7, and 9 ($P < 0.01$). iNOS: there were differences at days 3, 5, and 7 ($P < 0.01$). (Color version of figure is available online.)

atonia, the rat touches the water and wakes up. Control groups usually consist of either CC rats or rats placed onto a grid under the exact same environmental conditions.

It has been demonstrated that SD can bring about changes in behavior, emotion, cognition, and memory [17–19]. In addition, it can induce the secretion of hormones, the release of neurotransmitters, and alter the expression levels of some specific genes and proteins [2]. In our study, the rats in the SD group presented poor mental state and physical weak body after SD. The expression levels of estrogen increased with the duration of SD, reaching a peak on day 5 of SD, which corresponded with behavioral changes in the rats. Kawasaki *et al.* [33] observed that estrogen was involved in inflammatory reaction and the formation of estradiol—the major active form of estrogen—may enhance the expression of matrix metalloproteinases in synovial cells. Meanwhile, the level of estradiol in SD group decreased after fifth day and even got even lower than that of TC group on day 9. It may indicate that body adaptive response act to alleviate the damage after acute SD, and the detailed mechanism would be taken in the further experiments.

Ma *et al.* [26] observed that estrogen could activate the NF-κB pathway and therefore induce the increased expression levels of downstream target genes. Previous studies have revealed that estradiol aggravates synovitis of the TMJ and promotes the translocation of NF-κB p65; with the intervention of blockers of estrogen or NF-κB receptors, the aggravation of experimentally-induced synovitis of the TMJ was significantly alleviated [26]. The results of our study show that after SD, translocation of NF-κB p65 was evident in the synovial membrane. Taken together, these findings suggest that the NF-κB pathway may be activated by the increase of estrogen, and may be involved in SD-mediated synovitis of the TMJ.

Previous studies have shown that the concentrations of IL-1β, TNF-α [34], IL-6 [26], and IL-8 [35] were significantly higher in the synovial fluid of patients with TMD than in healthy subjects. The cytokine network plays an important role in TMJ inflammation. TNF-α, IL-1β, and IL-6 appear to be the major proinflammatory cytokines involved in TMJ pathology. [36] These cytokines can stimulate synovial cell proliferation and subsequent activation [34]. In addition, COX-2 is a key enzyme involved in the synthesis of prostaglandins, among which PGE2 in particular can contribute to joint pain [37]. iNOS is also involved in the pathologic process of inflammatory pain, and is associated with persistent inflammation and synovial membrane destruction in osteoarthritis [38].

The expression levels of TNF-α, IL-1, IL-6, IL-8, COX-2, and iNOS, which are pain-related factors, are regulated by the NF-κB pathway, and their levels have been shown to increase significantly when the NF-κB pathway is activated [13]. Our results showed that the expression of the pain-related factors IL-1β, IL-6, COX-2, and TNF-α significantly increased in the synovial membrane, which corresponded to the findings of the previous study [13]. The present findings therefore provide further evidence to suggest that estradiol-induced

potentiation of these cytokines in the synovial membrane results in aggravated TMJ inflammation. One interesting finding is that increasing level of IL-6, IL-1β, TNF-α, and iNOS dropped at ninth day after SD, but was still higher than normal. Meanwhile, the increasing level of estradiol in SD group alleviated from fifth day, which were even lower than TC group. This may indicate that body adaptive response act after acute SD and alleviate but does not clear the inflammatory reaction. Then the acute inflammatory reaction converted to a long-term inflammatory process, which plays a pivotal role in the development of TMD. The mechanism would be taken in the further experiments. Our data on the transcription of proinflammatory cytokines potentiated by estradiol further elucidate the proinflammatory effects of estradiol on joint inflammation and pain. Meanwhile, our results showed that the levels of proinflammatory facts by PCR were slightly elevated in TC group compared with the CC group (data not shown), which suggested that there was a baseline stress with the MMPM model. Similar to previous studies [29–31], MMPM could attenuate the effects of intervening stress in PS deprivation models, however, it has been shown that placement of rats on wide platforms induces some degree of PS deprivation.

Limitations of this study should be acknowledged. The MMPM method used in the present study is associated with less anxiety than the single platform method and other SD models. Nevertheless, it should be noted that increased psychological stress and muscle fatigue still occur under the multiple system and these should not be overlooked when interpreting our data.

5. Conclusions

SD can induce alterations in the serum concentration of estrogen and the TMJ microstructure, and increase the expression levels of the inflammatory cytokines TNF-α, IL-1β, COX-2, IL-6, and iNOS, which can induce inflammation of the synovial membrane in the TMJ. The NF-κB pathway has been implicated in the regulation of these inflammatory cytokines. SD therefore appears to play an important role in inducing TMD in rats. These results help us to understand why the prevalence, severity, and duration of TMD-related pain are greater in sufferers of agrypnia than in non-sufferers. Therefore, when treating TMD patients, especially those with sleep disorders, sleep should be taken into account and attempts should be made to improve patients' sleep.

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Authors' contribution: G.Z. and H.F. conceived and designed the experiments. G.W., L.C., C.M., Y.W., and Y.L. performed the experiments and acquired data. G.W., L.C., and Y.W. did the analysis and interpretation of data. G.W., G.Z., and H.F. wrote the article.

Disclosure

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in this article. There are no conflicts of interest in this article.

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